Integrating the Aquaculture of the green sea urchin *Strongylocentrotus droebachiensis* and the European oyster *Ostrea edulis* in the Gulf of Maine

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Abstract

The concept of integrated multi-trophic aquaculture is a growing trend in aquaculture systems design. It involves cultivating multiple species with complementary ecological requirements, so that all components have commercial value, as well as roles in biomitigation. This project aimed to apply the concept of multi-trophic integration to the culture of European oysters and green sea urchins.

The European Oyster (*Ostrea edulis)* was introduced to the Gulf of Maine in the 1940's as a compliment to the American oyster (*Crassostrea virginica)*. Wild populations are becoming highly conspicuous in the coastal zone, as its habitat does not overlap with *C. virginica*; an estuarine species. No aquaculture operations currently utilize this species. A fishery for the green sea urchin (*Strongylocentrotus droebachiensis*) was initiated in the Gulf of Maine in 1987, and quickly overfished. Populations show limited signs of recovery although the demand for urchin roe is high.

An innovative caging system for integrated culture of *S. drobachiensis* and *O. edulis* was developed and tested at a field site in Little Harbor, a part of Portsmouth Harbor, New Hampshire. The system was determined to be robust and potentially scalable for commercial culture. Free-roaming *S. droebachiensis* were observed to be attracted to the caging structure.

Preliminary testing occurred at a study site in Gosport Harbor, NH. *S. droebachiensis* was found to perform as a highly effective agent in bio-fouling mitigation. In cages containing juvenile *S. droebachiensis; O. edulis* recruits were more likely to survive and grow at a uniform rate in cages with reduced fouling.

Additionally, the solitary tunicate *Ciona intestinalis* was observed in high population densities on oyster caging equipment. *C. intestinalis* is a major contributor to bio-fouling in bivalve aquaculture operations worldwide. Dried samples were analyzed for nutrient composition, and considered as an alternative to wild finfish meal in animal feeds.

Introduction

The purpose of this project was to investigate the potential for integrated aquaculture of the European oyster (*Ostrea edulis),* and the green sea urchin (*Strongylocentrotus droebachiensis)*. *O. edulis* is a species of oyster native to Europe. It was artificially introduced as a compliment to the American oyster (*Crassostrea virginica*) in the 1940's. Since its introduction, natural populations have established themselves in coastal habitats from Rhode Island to Maine. There are no aquaculture operations currently utilizing the European oyster. However, wild populations in the southern part of the Gulf of Maine have increased in the coastal zone. The European oyster is now a prominent member of benthic habitats in protected areas along the coast (Harris, et al, 2013).

The demand for green sea urchin roe, known in restaurants as Uni, created a global fishery, which peaked briefly and rapidly declined to present low levels. While the demand for urchin roe is high, efforts to promote urchin aquaculture in the Gulf of Maine have not led to viable operations. Large-scale harvests of *S. droebachiensis* began in Maine in 1987 and peaked in 1993 at an estimated 19,050 metric tons. The fishery value peaked in 1995 at $35,604,275. (Fraungruber et al., in press). Ecological changes have contributed to a global decline in sea urchin fisheries since its peak in the 1990's. Since 2008 the annual catch in Maine has averaged 1,300 metric tons, with an economic value of $5.5 million (Fraungruber et al., in press). Fishermen and resource managers believe that wild stocks can and should be rebuilt for increased natural harvest. (Harris et al., 2003)

Observations of natural recruitment in the Gulf of Maine parallel the reduction in harvests. Large portions of urchin habitat have reverted to algal-dominated areas, which resist natural recruitment (Harris et al., 2003). There are however still areas in the Gulf of Maine that are potentially capable of supporting fisheries at greatly reduced levels (Harris et al., 2003). Stock enhancement through release of hatchery seed has been considered, but uncertainty regarding ecological and economic viability has discouraged public funding for sustained programs. (Fraungruber et al., in press) Implementation of culture systems investigated in this

study could lead to a viable system of stock enhancement, in an area that has seen a drastic reduction in both natural populations and fisheries.

A growing trend in aquaculture is integrating two or more species, complementary in ecological requirements, in a multi-trophic approach. While the demand for fish and shellfish continue to grow, many marine species are either in decline or failing to respond to management efforts (Harris et al., 2013; Barrington et al., 2009)

The green sea urchin and European oyster do not overlap in habitat and can both present new additions to the diversity of aquaculture in the Gulf of Maine. It is hypothesized that a benthic caging system can be developed that utilizes hatchery reared juvenile urchins to keep caged oysters clean. The cage system will provide a complex substrate that will protect the urchin in its juvenile stage, until it can be out-planted as a free roaming individual. The timing of out-planting will be determined by observing the size at which *S. droebachiensi*s becomes capable of boring into *O. edulis* shell, thus posing a threat to *O. edulis* survival. Out-planted *S. droebachiensis* will be free-roaming. However, the caging system will provide complex structure conducive to bio-fouling, acting as an attractant for free-roaming adult urchins.

Integrated multi-trophic aquaculture (IMTA) systems incorporate two compatible marine species; the cultivated species provides economic value while the extractive species provides a cost-effective form of waste removal. In this case, all components have commercial value. The hatchery will produce oyster spat in the summer and urchins during the winter. *S. droebachiensis* takes an average of three years to reach marketable size (50 mm) making monoculture operations economically challenging. (Swan, 1961) *O. edulis* reaches marketable size (35-50mm) in approximately two years, providing more immediate returns on investment. (Hidu & Lavoie, 1991) Market size *O. edulis* are traditionally harvested in late summer, while *S. droebachiensis* roe is harvested at peak quality during winter months.

Two locations, similar in benthic community and structure, were selected for experimentation with this integrated system. In Gosport Harbor, Isles of Shoals, *S.*

droebachiensis was preliminarily tested as an anti-bio-fouling agent. After recruitment occurred in both species, differences in growth and survival were compared between urchin and no-urchin treatments. In Little Harbor, New Castle, New Hampshire, a revised benthic caging system utilized modified commercially available SEAPA oyster baskets. Growth and survival of *O. edulis* was quantified between urchin and no-urchin treatments.

To determine the potential stocking capacity of SEAPA cages, multiple stocking densities for *O. edulis* were tested. Mortality was recorded from February to April, 2014.

Although there is no law preventing farmers from using unsubmerged equipment, permitting of non-benthic operations is very challenging due to multiple user conflicts in coastal waters.

Additionally, high-density colonization of the solitary tunicate *Ciona intestinalis* was observed on oyster cages at the UNH Coastal Marine Lab in Newcastle, New Hampshire. *C. intestinalis* has a worldwide distribution, and has been documented as a major pest in bivalve aquaculture operations in the Gulf of Maine. (McKindsey, 2012) Considering the rapid growth in the farmed fish and shrimp industries since the 1990's, the current industry trend of using increasing amounts of wild-caught fish to feed farmed species is highly unsustainable. Production of a single kilogram of farmed species typically uses two to five kilograms of wild-caught fish processed into fish meal and fish oil for feed. (Naylor et al., 2000) The need for alternative sources of marine protein is insistent, as wild stocks are already substantially reduced. Due to a high level of fecundity and a wide host range *C. intestinalis* is a potentially exciting match for this role. Researchers at the University of Bergen, Norway, have reported 55% crude protein content in dried specimens. (Uni Research, 2013)

Local samples were collected from submerged oyster cages at the University of New Hampshire, Coastal Marine Lab, located in New Castle, New Hampshire. Samples were subjected to proximate nutrient analysis to compare protein and fat composition to those found in Norwegian waters.

Methods

Study Sites

Two sites were selected for field studies. Preliminary recruitment studies were conducted in Haley's Cove within Gosport Harbor at the Isles of Shoals, New Hampshire. (Figure 1) An analysis of the revised caging system was conducted in Little Harbor; a part of greater Portsmouth Harbor, New Hampshire. (Figure 2)

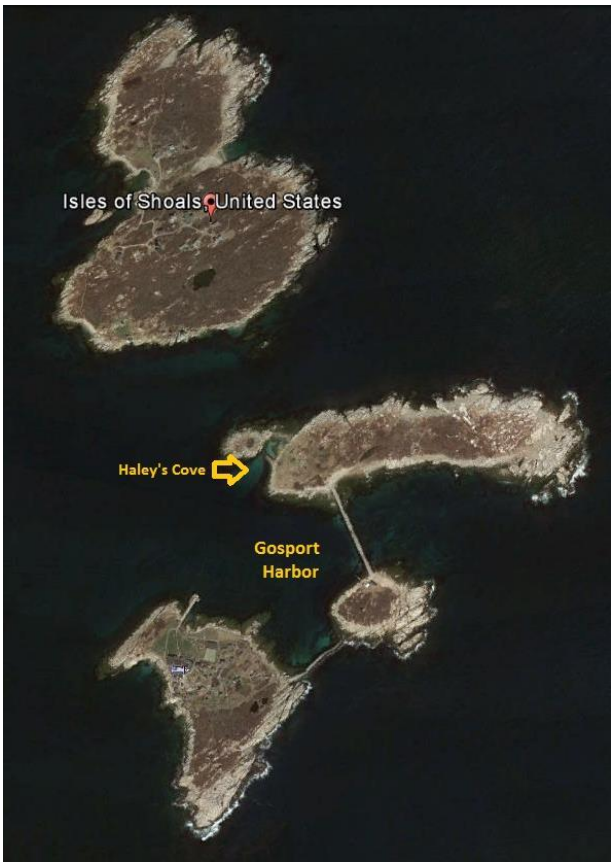

Figure 1: Google Earth map depicting Haley's Cove, Gosport Harbor study site, Isles of Shoals, NH.

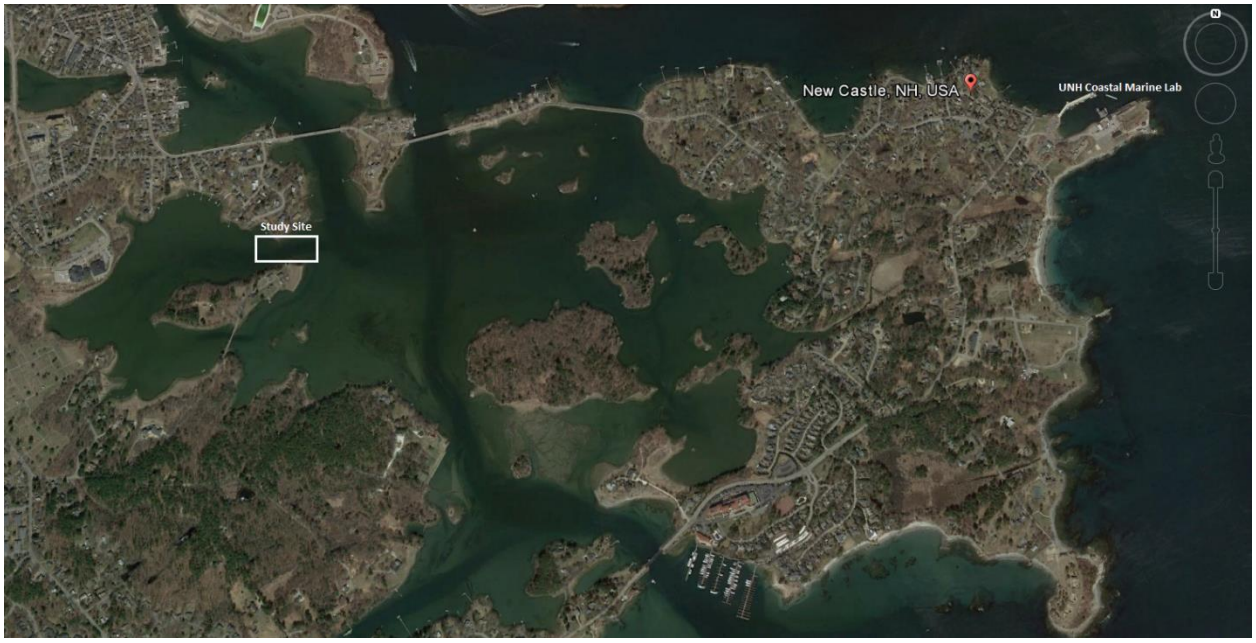

Figure 2: Google Earth map denoting the locations of the UNH Coastal Lab and the Little Harbor study site. Site is a well-protected channel with cobble and shell hash bottom that grades into mud as you approach the shoreline. Water depth is approximately four meters at high tide, and one meter at low.

Revised Caging System Analysis: Little Harbor

Caging System

Oyster cages were purchased from SEAPA Pty Ltd. Components included: Size 12x1100mm basket mesh (Code 12-1100 MP), Size 12mm standard end cap (Code 12MPEC), and Size 12mm auto cap (Code 12MPAC). (Figure 3) Ten cages were assembled and arranged into two strings of five connected with 3/8" polypropylene rope. Rope ran through the end caps and eyeholes on the top of the cage. Cages were prevented from sliding along the rope with simple knots tied on the outer edge of each end cap. Thirty feet of rope was run between each cage. This was to allow for easier manipulation of heavy cages by allowing one cage to sit on the boat deck whilst the others' could be left on the seafloor.

Each cage was retrofitted with two marine grade bricks, and a 1.2x.75 meter sheet of plasticized wire mesh. (Figure 3) Heavy-duty nylon cable ties were used for easy attachment. A number of drop tests into moving water were performed to determine this construction's ability to land "upright" regardless of entrance angle and flow direction.

A single buoy line was fastened to an end cage on each string allowing for hooking and easy retrieval. Each string of cages was dropped in a way that maximized rope-length between individual cages, minimizing risk of tangle. The string with urchins was deployed parallel to the northern edge of the channel approximately ten meters across from the no-urchin string parallel to the southern edge.

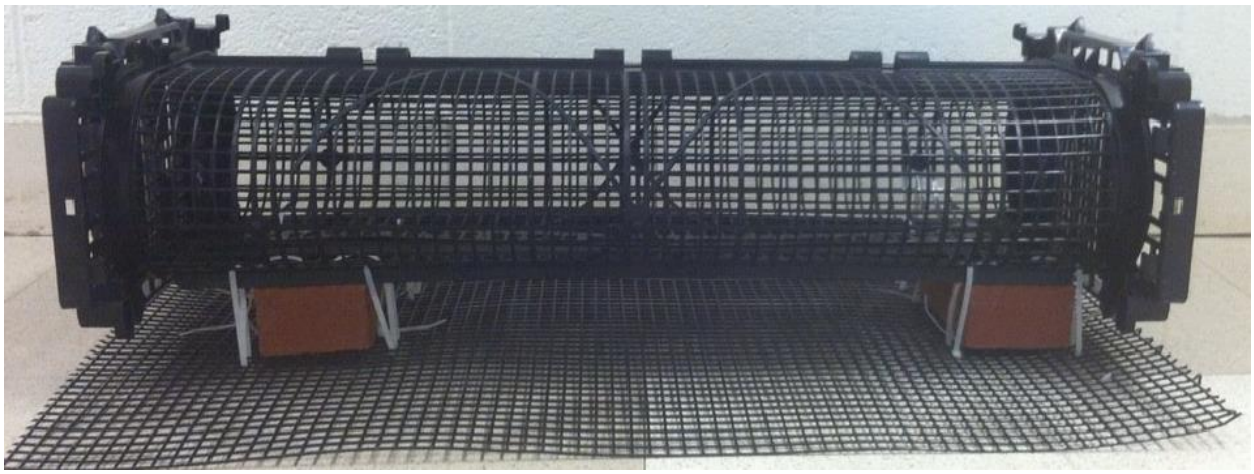

Figure 3: Sample cage construction

Organism Procurement

S. droebachiensis were hatchery raised and obtained from the UNH Coastal Marine Lab. *O. edulis* were collected from wild populations occurring near the Isles of Shoals during November, 2013. Specimens were held and prepared for deployment at the UNH Coastal Marine Lab, New Castle, NH. Holding tanks were supplied with constantly circulating seawater from the adjacent bay.

S. droebachiensis specimens were selected to be roughly the diameter of a quarter dollar (24mm) and stocked at a rate of twenty-five per cage in one string of five cages.

O. edulis were scraped clean of fouling organisms and smaller size classes were selected preferably. Interconnected oysters were pulled apart if possible, and conglomerates were not used. *O. edulis* were stocked at a rate of twenty-five per cage into all ten cages.

Data Acquisition

The contents of each cage were emptied and photographed prior to deployment. (Figure 4) Care was taken to prevent oyster shell overlap so that accurate size analysis could be performed. Photographic data was collected every three to five weeks by hauling each cage onto the boat and laying out all specimens in a trough alongside a ruler. Mortality was recorded and photographed accordingly. Data collection occurred from November 23, 2013, to April 9, 2014.

Photographic data was analyzed using Photoshop CS5's Magnetic Edge Finder. (Figure 5) In order to most accurately outline each oyster the tool was set to survey a 5 pixel width, detect 5% contrast, and plot points at a frequency of 100 per unit. The unit of measurement was automatically converted from pixels to centimeters in Photoshop by identifying a one centimeter length on the ruler included in each photograph. After all oysters were measured the data was imported into Microsoft Excel. Total oyster surface area in each photograph was quantified and compared between dates.

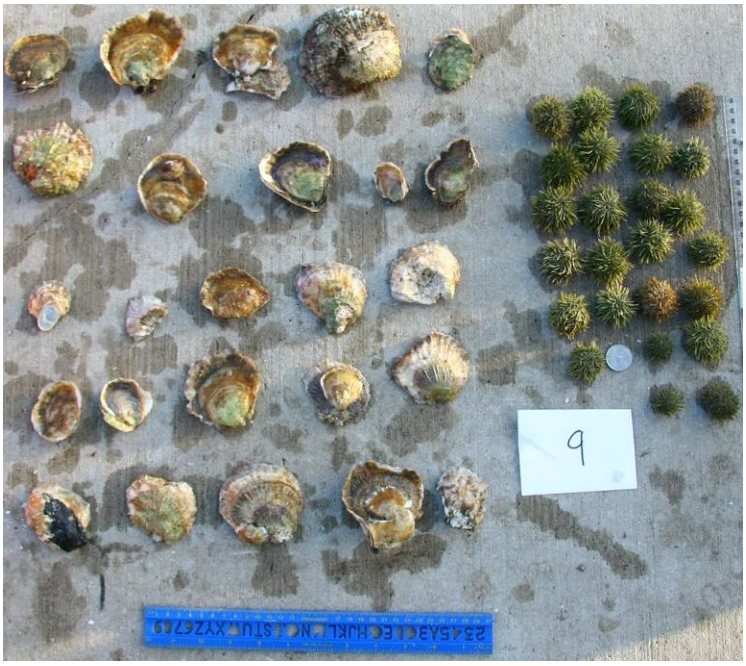

Figure 4: Sample data acquisition photograph for cage number 9. This photo was taken prior to deployment while cages were being stocked in preparation for transport to the Little Harbor site.

Figure5: Partially processed image in Photoshop. The black filled oysters have been outlined using the magnetic edge finder and filled to denote they have already been measured. The ruler was used to set the scale in each image.

Stocking Density Evaluation

Three SEAPA cages were stocked with thirty, fifty, and one-hundred mid-size to adult oysters. The cages were hung horizontally from the UNH research pier at the Coastal Marine Lab in Newcastle, NH. Mortality was recorded monthly from February 2, 2014 through April 2, 2014.

Recruitment Study: Haley's Cove, Gosport Harbor

Experimental Design

Six (40x40x14cm) cages were constructed from plasticized wire and deployed at Haley's Cove in Gosport Harbor, Isles of Shoals, New Hampshire. Deployment occurred on December 7, 2012 and cages were collected on August 23, 2013. Three cages were deployed with *S. droebachiensis* and *O. edulis*, and three cages contained only *O. edulis*. (Figure 6) After summer recruitment occurred for both species recruits were counted in all cages. Growth and survival was recorded for all *O. edulis* spat. *O. edulis* spat growth was measured as length from the umbo to the farthest edge of the shell. Mortality in both species was recorded. Statistical analysis was performed using JMP statistical software.

Figure 4: Initial cage format. The left image contains O. edulis and S. droebachiensis, the right contains solely O. edulis.

Ciona intestinalis Nutrient Analysis

Live *Ciona intestinalis* specimens were collected from New Castle Harbor, New Castle, NH on January 20, 2014. Seven healthy individuals were oven dried overnight and sent to New Jersey Feed Labs for proximate testing (moisture, protein, fat, fiber, ash).

Results

General Observations

In the Little Harbor channel; free-roaming *S. droebachiensis* were observed to colonize the outside of cages as well as the base structure. (Figure 7) *O. edulis* were observed to be cleaner in cages containing *S. droebachiensis.* The revised caging system proved to be robust and manageable.

Figure 7: Three free-roaming urchins that had colonized the outer walls of one cage structure.

At the Haley's Cove recruitment study site *O. edulis* spat only settled on other *O. edulis* shell. A preference between settlement on live or dead *O. edulis* could not be determined by our study. However, zero *O. edulis* settlement occurred on American oyster (*Crassostrea virginica*) cultch stocked in the cages.

Upon collection the Haley's Cove cages originally stocked with urchins showed significantly reduced biofouling compared to the no-urchin treatment. (Figure 8)

Figure 8: Cage's collected from Haley's Cove. (Above) A cage with no-urchins. Conspicuous colonization of Gracillaria and Ulva algal species. Cage sides and oyster shells have high densities of various fouling organisms. (Below) A cage stocked with urchins. Fouling organisms are in comparatively very low densities on sides of cage. Shells are relatively devoid of colonizing organisms.

Quantitative Results:

Revised Caging System Analysis: Little Harbor

A 0.8% difference in *O. edulis* mortality was detected during the study period. (Table 1)

Table 1: Total O. edulis mortality expressed as an individual count, and percentage of total O. edulis in each treatment. There were 125 O. edulis in each treatment.

Treatment	Mortality	Percent
Urchins	**2**	**1.6%**
No-Urchins	**3**	**2.4%**

Recruitment Study: Haley's Cove, Gosport Harbor

The number of *O. edulis* recruits was compared between urchin and no-urchin treatments. Comparison of the average number of recruits, and recruit survival, showed slight variation between treatments. *O. edulis* recruits averaged 6% more likely to survive in the urchin treatment. (Table 2)

Table 2: The number of initially stocked and post-recruitment O. edulis and S. drobachiensis individuals in each cage as well as number of deaths. Cages 1-3 were deployed with urchins. Cages 4-6 deployed with no-urchins. Cage 4 showed the lowest percent oyster recruit survival (33%), it was deployed without urchins and zero urchins recruited into the cage.

Cage	Urchins	Urchin Recruits	Dead Oysters	Live Oysters	Oyster Recruits Total	Oyster Recruits Surviving	Percent Oyster Recruit Survival
1	32	1	12	4	30	19	63%
2	27	3	7	9	24	16	67%
3	31	1	9	7	14	10	71%
4	0	0	12	4	24	8	33%
5	6	6	8	8	21	13	62%
6	6	6	8	9	30	26	87%
Urchins	30.00	1.67	9.33	6.67	22.67	15.00	67%
No-urchins	4.00	4.00	9.33	7.00	25.00	15.67	61%

A two-sample T-test assuming equal variance was performed comparing *O. edulis* spat length between urchin and no-urchin treatments. Statistically significant differences in *O. edulis* spat length were not detected between treatments. (P-value=0.03; 95% confidence interval) However, variation in spat size was much greater in the no-urchin treatment. (Figure 9)

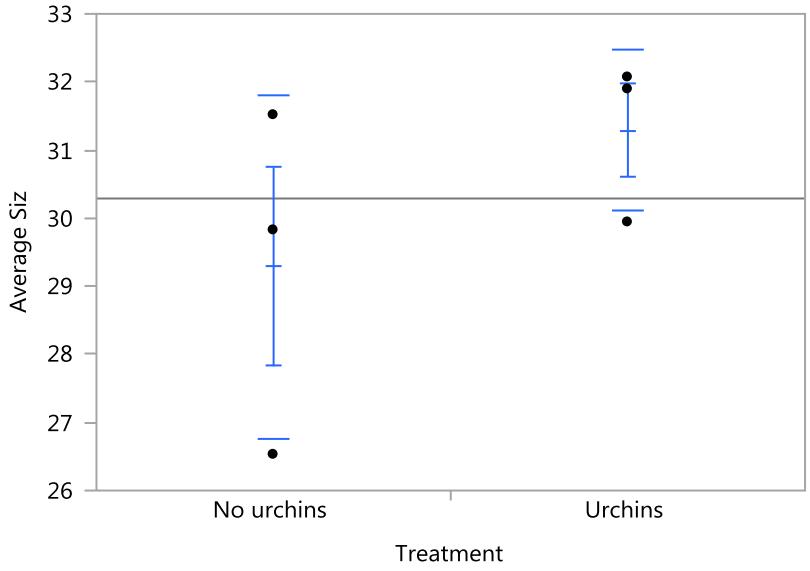

Figure 5: One-way analysis of average size (spat length) by treatment. Far greater size variation can be observed in the "no-urchins" treatment. No-urchin treatment had a standard deviation of 2.53mm while the urchin treatment deviated by 1.19mm.

Stocking Density Evaluation

O. edulis deaths occurred in cages stocked with 50 and 100 individuals. The SEAPA cage stocked with 30 oysters exhibited zero mortality. (Table 3)

Table 3: The number of O. edulis individuals stocked in each one of three cages compared to the total mortality counts over a three-month period. Column three shows that O.edulis experienced highest percent mortality when stocked fifty per cage, however highest mortality count occurred in the one-hundred oyster treatment.

Number Stocked	Total Mortality	Percent
30	0	0.0%
50	2	4.0%
100	3	3.0%

***Ciona intestinalis* Nutrient Analysis**

Figure 4 shows the averaged composition of *C. intestinalis* over seven dried samples. THE FIGURE NEEDS TO BE REVISED TO GIVE COMPONENTS AND VALUES

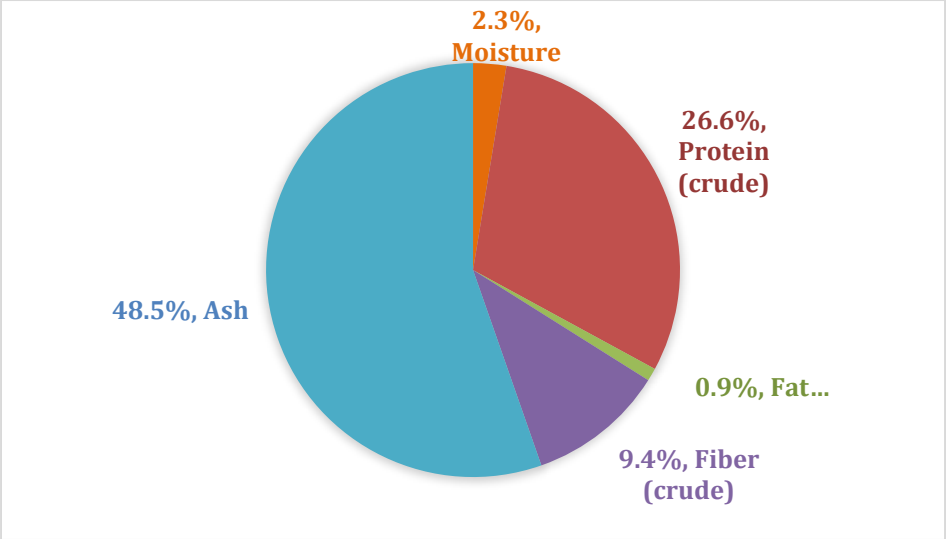

Figure 6: Average percent nutrient composition of C. intestinalis. Crude protein and fat accounted for 27.5% of C. intestinalis dried mass.

Discussion

The benefits of an integrated approach to culturing European oysters (*Ostrea edulis)* and green sea urchins (*Strongylocentrotus droebachiensis*) are yet to be fully understood. As natural grazers, green sea urchins remove sessile fouling organisms from substrata (Barrington et al., 2009). Numerous studies have been performed to test methods of inhibiting or minimizing bio-fouling organisms on Mollusca. A study testing various organisms' efficiency at the biological control of fouling on scallop cultivation revealed that sea urchins were most effective, and could thus be commercially exploited alongside scallops in a form of polyculture (Ross et al., 2004). A study in British Columbia determined that *S. droebachiensis* acted as an effective and economical form of biocontrol in sablefish net pens. (Edwards, 2008) Although our data was statistically inconclusive, continued analysis of the modified SEAPA caging system through the summer months should yield better results due to the seasonality of oyster growth.

Revised Caging System Analysis: Little Harbor

Thus far, the Little Harbor based revised caging system analysis has proven untimely; apex growth rates are expected from late spring, when gonad development occurs, through fall (Boghen, 1995). Optimum growing temperatures for *O. edulis* fluctuate near 18°C (Toba, 2002). However the average water temperatures throughout the duration of the revised caging experiment fluctuated near 2°C and below. Therefore, it is not surprising that *O. edulis* growth could not be observed during the study period.

Qualitatively, cages containing *S. droebachiensis* were observed to contain *O. edulis* with less algal coverage than those without urchins. This result confirms observations from Haley's Cove where cages containing *S. droebachiensis* had relatively algae free *O. edulis*. Further study of the effects of biomitigation by *S. droebachiensis* on *O. edulis* may provide insight on the cause of the observed variation in *O. edulis* mortality between urchin and no-urchin treatments. (Table 1)

Furthermore, the objective to design a productive, robust, and cost-effective caging system was met with our modified SEAPA oyster basket design. The cost to construct our system was comparable to the cost of purchasing commercially available oyster bags and "condo" racks for benthic culture. Observations from the Little Harbor site showed that the system was able to withstand strong currents associated with major storm events without loss of product. Furthermore, free-roaming wild *S. droebachiensis* were observed to colonize the caging structures. This result is consistent with observations by Dumont et al. (2007) stating that sea urchins tend to aggregate around food sources, which can provide cover for the juveniles. At the Little Harbor site, the seafloor is relatively devoid of complex structure allowing for bioaccumulation. Therefore, we can hypothesize that most out-planted urchins would aggregate on caging structures as was observed in wild populations, due to the presence of a high volume food source.

Recruitment Study: Haley's Cove

Although statistically significant variation in survival and growth of *O. edulis* was not detected between urchin and no-urchin treatments, qualitative results from this study demonstrated proof of concept, and inspiration for further investigation. Table 2 shows that cages deployed with *S. droebachiensis* had a 6% higher rate of survival in *O. edulis* recruits, consistent with observations of adult *O. edulis* survival at the Little Harbor site. Additionally, Figure 9 shows that *O. edulis* recruits grown with adult urchins present had less variation in growth rate than those growth without urchin presence. Uniform growth rate is a highly desirable characteristic in aquaculture.

By observing photographs of cages with and without *S. droebachiensis* (Figure 8) we can observe far lower density populations of sessile invertebrates and algae in cages deployed with *S. droebachinsis*. It can be hypothesized that increased water flow through cage walls, as well as increased suspended particulate matter from urchin feeding may promote the observed health and uniform growth of *O. edulis* spat.

Stocking Density Evaluation

Little variation in survival of *O. edulis* was detected between the 30, 50, and 100 individual treatments. No mortality occurred in the 30 individual treatment, and the 100 individual treatment showed the greatest net mortality at 3%. However, it would be advisable to increase the number of replicates, variation in stocking densities, and duration of this experiment. *O. edulis* experiences the highest rates of mortality during the summer months. (Carlucci et al., 2010, Jarayabhand, 1988) Therefore, our wintertime data may not be indicative of losses incurred during a full grow-out. Additionally, Carlucci et al. (2010) confirmed our observed lack of variation in mortality rates at the tested densities, their study found no significant variation in mortality between *O. edulis* stocked at 40 and 90 individuals per bag. However, they did find significant variation in growth rates.

Ciona intestinalis Nutrient Analysis

C. intestinalis harvested during the month of January was determined to contain an average of 26.6% crude protein. Commercial fish meal for carnivorous species is usually marketed with 57-77% crude protein. (Miles & Jacob, 1997) Large-scale research investigating the efficacy of intensive *C. intestinalis* culture is currently underway at the University of Borgen, Norway. They have reported crude protein content of 55%, an anticipated crop of 100-200kg per square meter. (Uni Research, 2013)

If we were potentially able to harvest 150kg per square meter of live *C. intestinalis*, and then press out 95% of its water content we would be left with 7.5 kg of dried biomass. From this biomass and our crude protein content of 26.6% we could theoretically harvest 2.1 kg crude protein per square meter. Therefore, to produce 1 U.S ton of crude protein we would cultivate 3,410kg live *C. intestinalis* on approximately 23 square meters of substrate. Further research into the possibility of producing these theoretical cultures in the Gulf of Maine is advisable. Further sampling of *C. intestinalis* nutrient content during the spring reproductive period and summer months may also reveal increased protein and fat concentrations.

Overall, further investigations into the harvest of *C. intestinalis* as an alternative source of marine protein may result in a better understanding of its economic viability.

Conclusions

Proposed Modifications to the Revised Caging System

The experimental lease site could be selected to avoid strong flows during storms. Given the vulnerability of the current site location, exposure to storm events have the potential to adversely affect benthic caging systems. Storms have a negative effect on aquaculture systems because strong currents bring sediment and excess nutrients into the sample site. Sediment loading causes turbidity, leading to the reduction of light penetration, and the increase of nutrient levels, thus the formation algal blooms, which deplete dissolved oxygen concentrations. Heightened

sediment levels have the potential to smother benthic aquaculture systems further reducing dissolved oxygen content (Deel, 2011). Furthermore, forceful currents displaced the cages and caused them to intertwine, which increased time and labor during data collection.

In terms of cage setup, installing a more robust connection between the cage, the brick, and the plasticized wire mesh would enhance the design. The original cage design is subject to disfigurement due to the nylon cable ties. A more durable connection such as a wire cable would be able to withstand degradation from prolonged exposure to currents. Additional adjustments include shortening the distance of polypropylene rope in between each cage. The 30-foot distance between each cage proved to be excessive and had adverse effects on cage deployment. Due to the compacted area of the research vessel Gaylen J, it was difficult to determine the succession of the cages with surplus rope aboard, which additionally lead to greater entanglement. Cage deployment may also benefit from clustering cages, as opposed to a linear formation, by fastening multiple cages onto one piece of plasticized wire mesh. The increased weight would promote stability in the water, while also maintaining manageability and reducing deployment time.

Proposed Recruitment Study Alterations

The intention of the Little Harbor recruitment study was to provide verification of increased numbers of oyster recruitments and uniform expedited growth rates due to the presence of *S. droebachiensis* as an anti-fouling agent. This study provided photographic evidence depicting that *S. droebachiensis* presence significantly diminished bio-fouling in oyster cages. Data revealed that those oysters in the presence of urchins had a slight advantage with an increased oyster recruit survival rate of 7%, and little variation in growth rate. This data however was not statistically significant; thus, future trials are suggested with modifications including increased replication within treatments.

Suggested Alterations to the Stocking Density Evaluation:

When furthering research upon oyster cage stocking densities, it would be beneficial to increase replicates. To improve statistical significance, greater than one cage of each density (30, 50, 100) would be necessary with a suggested increase of five cages per density. Cages having densities greater than 100 oysters per cage should be explored in order to obtain a maximum density that does not inhibit growth rates to achieve peak output from the system. Varying urchin densities should also be investigated for comprehension of urchin growth rate affected by density, and maximum bio-fouling remediation potential.

Furthering *C. intestinalis* Research:

An additional *C. intestinalis* feasibility study would be able to determine how much *C. intestinalis* could be produced as well as how protein and lipid contents vary with seasonal reproductive cycles. Test substrates should be deployed in New Castle Harbor to ascertain *C. intestinalis* settlement and production volumes. In a study by Reinhardt et al. (2012), tunicates were successfully cultivated using both a natural rock wall and roughened polyvinyl-chloride (PVC) panels as substrates. Valentine et al. (2007) recorded tunicate colonization using clear plastic containers with perforated sides for water circulation and covered in plastic mesh as an additional substrate to attach onto. Other substrates that have been identified as successful for tunicate colonization include floating eelgrass or macroalgae (Carver et al., 2006) Natural and artificial substrates should be explored, as well as existing infrastructure that could be repurposed.

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